American Journal of Medical Genetics (Neuropsychiatric Genetics) 67:264–288 (1996)

Polygenic Inheritance of Tourette Syndrome, Stuttering, Attention Deficit Hyperactivity, Conduct, and Oppositional Defiant Disorder: The Additive and Subtractive Effect of the Three Dopaminergic Genes—DRD2, DβH, and DAT1

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Polymorphisms of three different dopaminergic genes, dopamine D_2 receptor (DRD2), dopamine β-hydroxylase (DβH), and dopamine transporter (DAT1), were examined in Tourette syndrome (TS) probands, their relatives, and controls. Each gene individually showed a significant correlation with various behavioral variables in these subjects. The additive and subtractive effects of the three genes were examined by genotyping all three genes in the same set of subjects. For 9 of 20 TS associated comorbid behaviors there was a significant linear association between the degree of loading for markers of three genes and the mean behavior scores. The behavior variables showing the significant associations were, in order, attention deficit hyperactivity disorder (ADHD), stuttering, oppositional defiant, tics, conduct, obsessive-compulsive, mania, alcohol abuse, and general anxiety—behaviors that constitute the most overt clinical aspects of TS. For 16 of the 20 behavior scores there was a linear progressive decrease in the mean score with progressively lesser loading for the three gene markers. These results suggest that TS, ADHD, stuttering, oppositional defiant and conduct disorder, and other behaviors associated with TS, are polygenic, due in part to these three dopaminergic genes, and that the genetics of other polygenic psychiatric disorders may be deciphered using this technique.

KEY WORDS: polygenic, attention deficit hyperactivity disorder, ADHD, conduct disorder, Tourette syndrome, dopamine, DRD2, DβH, DAT1, transporter

INTRODUCTION

Tourette syndrome is a complex neuropsychiatric disorder characterized by chronic motor and vocal tics and a wide range of associated behaviors including alcohol and drug abuse, depression, and obsessive compulsive, attention deficit hyperactivity, conduct, sleep, learning, sexual, and anxiety disorders [Comings and Comings, 1987, 1993; Comings, 1990a, 1995e]. While the TS is usually assumed to be inherited as an autosomal dominant trait [Comings et al., 1984; Pauls and Leckman, 1986], linkage studies have excluded virtually the entire genome without finding the *Gts* gene [Heutink et al., 1992; Tsui, 1994]. Because of the wide spectrum of associated disorders and the evidence that *Gts* genes are inherited from both parents [Comings et al., 1989; Comings, 1990a; Kurlan et al., 1994a], we have suggested TS is a polygenic disorder and that these genes involve the metabolism of dopamine, serotonin, norepinephrine, and other neurotransmitters, with each gene contributing only 1 to 10% of the variance [Comings, 1995d,f, 1996]. Numerous lines of evidence suggest that defects in the metabolism of dopamine are involved in TS [Comings, 1990a]. In previous studies we have reported an association between TS and ADHD and the dopamine D_2 receptor gene (DRD2) [Comings et al., 1991; Comings, 1992]. These combined observations have led us to test the polygenic hypothesis by examining the potential ad-

Received for publication February 22, 1995; revision received October 19, 1995.

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ditive effect of three dopamine genes, DRD2, dopamine β-hydroxylase (DβH) and the dopamine transporter (DAT1) on a number of behavioral variables in TS probands, their relatives, and controls.

Dopamine D_2 Receptor Gene

In 1990 Blum et al. [1990] reported a strong association between alcoholism and the *Taq* I A1 allele of the DRD2 gene. Subsequent studies have given mixed results with some failing to confirm this association [Bolos et al., 1990; Gelernter et al., 1991] while others have supported it, especially with more severe forms of alcoholism [Noble, 1993; Blum et al., 1995]. The significant association between TS and alcoholism [Comings, 1990a, 1994c] and the effectiveness of dopamine D_2 antagonists in the treatment of TS, led us to examine the hypothesis that the D_2A1 allele might be more highly associated with a spectrum of impulsive, compulsive, addictive behavioral disorders, including TS, than alcoholism per se.

In our initial study [Comings et al., 1991], we observed the D_2A1 allele in 24.5% of 314 non-Hispanic Caucasian controls vs. 44.9% of 147 TS subjects, $P < 0.0001$; 46.2% of 104 ADHD subjects, $P = 0.0001$, and 54.5% of 33 subjects with autism, $P = 0.0005$. In a subset of 39 subjects with severe TS, the D_2A1 allele was present in 59%, $P < .0001$. Devor [1992] reported a prevalence of the D_2A1 allele was 31.3% of TS cases with comorbid obsessive-compulsive behaviors or ADHD, versus 20.0% for those without comorbid conditions. We reported additional studies showing a progressive increase in the prevalence of the D_2A1 allele from 35.0% in 20 subjects with mild TS, to 40.4% in 146 subjects with moderate TS, to 55.5% in 54 subjects with severe TS [Comings, 1992].

Gelernter et al. [1992] reported a prevalence of the D_2A1 allele of 31.8% in 22 subjects with chronic motor tic disorder and 45.8% in 59 subjects with TS. If we equate our mild TS subjects to their chronic motor tic disorder, and our moderate and severe TS to their TS subjects, the results of the two studies are virtually identical, 31.8 and 35.0% for mild-CMT, and 45.8 and 44.5% for TS per se. Gelernter et al. [1994b] subsequently reported a study of 64 TS or CMT subjects from 4 large pedigrees. When the frequency of the D_2A1 allele was examined in these subjects, stratified by two different measures, there was no association between the D_2A1 allele and tic severity. However, examination of the prevalence of the D_2A1 allele in the four pedigrees averaged 51.6%. When compared to the prevalence of the D_2A1 allele of 25.9% in the total reported 714 non-Hispanic Caucasians screened for drug and alcohol abuse [Comings et al., 1996c], $P = 0.000012$. We pointed out [Comings, 1995b] that our stratification of cases was by a global rating of severity. When we stratified by tic severity we also found no correlation with the D_2A1 allele. While Gelernter et al. [1995] suggest that the high prevalence of the D_2A1 allele in these 64 TS cases could have been a result of a founder effect artifact in the four families, it is also possible that the high incidence of TS in the families was due, in part, to the high frequency of the D_2A1 allele (see Discussion).

The most recent study of the D_2A1 allele in TS was by Nöthen et al. [1994]. They examined a total of 61 TS subjects and found a D_2A1 gene frequency of .22 or prevalence of 37%. While there was no correlation with severity, using a grading system similar to ours, the number of severe cases was relatively small (6). They also used the potentially powerful haplotype relative risk method [Falk and Rubinstein, 1987] and found no difference between the frequency of the D_2A1 allele in TS subjects (.22) vs. parental control alleles (.22). However, an analysis of the haplotype relative risk technique where the frequency of the test allele, in a two allele system, is .1 to .2, shows that only 32 to 48% of the paternal mating types are uninformative. This, plus the fact that the purported effect of the D_2A1 allele is quite modest (odds ratio of 2 to 3), and the fact that the DRD2 locus is probably only one of a polygenic set of genes in TS [Comings, 1996], suggests that well in excess of 200 informative parent child sets, or over 400 total sets, or over 1,200 total DNA samples would have to be studied to provide the power necessary to unambiguously exclude a role of the D_2A1 allele [Comings, 1996c]. Studies of severely affected probands versus unaffected controls of the same racial and ethnic background are more powerful in the sense that they require far fewer subjects.

Since our initial reports we have examined a total of 274 TS probands. Of these 40.1% carried the D_2A1 allele. When compared to the 714 known non-Hispanic Caucasian controls, $P = .000012$. When the total non-overlapping cases from the above studies are summed, a total of 432 non-Hispanic Caucasian TS probands have been tested with a D_2A1 allele prevalence of 40.7%. Compared to the 714 known non-Hispanic Caucasian controls, $\chi^2 = 27.43$, $P = .00000016$. The prevalence of the D_2A1 allele in the 158 TS cases reported by others (41.7%) is virtually identical to those reported by us (40.7%). Despite the lack of correlation between the D_2A1 allele and tic severity in all four of the relevant studies reviewed above, three studies found a correlation when using a more global assessment of severity. This suggests there may be an association between the DRD2 locus and non-tic, comorbid behavioral aspects of TS. Thus, we sought to reexamine the association between the D_2A1 allele using TS probands, their relatives, and controls. We were especially interested in the following questions: 1) Is the D_2A1 allele associated with measures of severity for comorbid behaviors independent of the tics? 2) If 13 probands, their relatives and controls are examined using a structured instrument to assess a variety of these comorbid behaviors, is there a progressive increase in prevalence of the D_2A1 from controls without those behaviors, to TS subjects or relatives without those behaviors, to TS subjects or relatives with those behaviors? 3) Are the means of the different symptom scores significantly different for those carrying vs. those not carrying the D_2A1 allele? 4) If the controls are left out of the analysis is there still a significant difference between the means of any of the behavioral scores in D_2A1 allele carriers versus noncarriers? 5) If the characteristic of polygenic inheritance is the requirement for several genes to produce a

significant effect on the phenotype, is the effect of the D_2A1 allele additive to the effect of alleles of other dopaminergic genes, such as the dopamine β-hydroxylase (DβH) and the dopamine transporter (DAT1) genes?

Dopamine β-Hydroxylase

DβH is one of the major enzymes for dopamine metabolism and catalyzes the conversion of dopamine to norepinephrine. It is localized to chromosome 9q34, closely linked to the ABO blood group and arginosuccinate synthetase loci (theta = 0) [Perry et al., 1991; Wilson et al., 1988]. Plasma DβH levels are genetically controlled and stable over time in older children and adults [Faraone et al., 1994; Vuchetich et al., 1991; Weinshilboum, 1983; Weinshilboum et al., 1973]. Through feedback inhibition, norepinephrine inhibits tyrosine hydroxylase, which in turn inhibits the production of dopamine and norepinephrine [Anden et al., 1973; Axelrod and Weinshilboum, 1972]. In experimental animals, the inhibition of DβH activity results in a decrease in norepinephrine levels which releases the inhibition of tyrosine hydroxylase resulting in the excessive production of dopamine. The latter is associated with hyperactivity, aggression, self-stimulation, and stereotypic movements [Randrup and Scheel-Kruger, 1966].

These interrelationships suggest a possible role of DβH in aggression, ADHD, and conduct disorder (CD) in humans. Quay [1986] proposed that externalizing disorders such as conduct disorder were associated with a decrease in noradrenergic function and an increase in dopaminergic function, a pair of conditions that would be uniquely brought about by a DβH deficiency. In this regard, Rogeness and colleagues have reported an increased frequency of the diagnosis of CD in emotionally disturbed boys with low plasma DβH levels [Rogeness et al., 1984, 1986, 1987, 1988]. In one study they examined plasma DβH levels in 420 boys admitted to a children's psychiatric hospital. Using 6 mUM/min/L as a cut off for "near zero" levels of DβH, they found that 54% of the boys with near-zero DβH were given a diagnosis of CD, undersocialized, and 24% were given a diagnosis of borderline personality disorder, compared with 21% and 10% respectively for the children with levels ≥15 uM/min/L. In other independent studies of hospitalized patients, Galvin et al. [1991] also found low DβH levels in CD. By contrast, in outpatient studies at a juvenile detention center [Pliszka et al., 1988b] and a community mental health center [Pliszka et al., 1988a] an association between CD and plasma DβH was not found. However, an outpatient study by Bowden et al. [1988] found that low DβH levels were much more likely in ADHD children who also had CD than ADHD children without CD.

To examine the possibility that the use of a continuous scale rather than dichotomous present or absent diagnostic categories would be more productive, Gabel et al. [1993] examined a group of boys entering an inpatient diagnostic center. Using the Parent Achenbach Child Behavioral Checklist (CBCL) for children under 12 years of age there was a significant inverse correlation between the DβH and scores for social problems, delinquent behavior, aggressive behavior, and the externalizing score, but not with other scores such as anxious depressed and internalizing score. By contrast, for teachers ratings after 1 month in the residential treatment setting, there were significant positive correlations with scores for withdrawn, anxious depressed, social problems, internalizing score, and aggressive behavior. Except for the aggressive behavior, this was consistent with the findings of Rogeness and co-workers of a positive correlation of high DβH levels with dysthymic disorder and separation anxiety disorder [Rogeness et al., 1988]. However, the Gabel et al. [1993] results are complicated by the fact that there was an inverse correlation between parent and teacher scores. In the older group of boys ≥12 years of age, there was a significant inverse correlation only with social problems, and no significant correlations with the Teacher ratings. Galvin et al. [1991] also found an inverse correlation between DβH and possible or definite neglect or abuse before 36 months of age. While they suggested that low DβH may be a biological sequelae of seriously disrupted attachment, it could also simply represent the inheritance of low DβH genes from abusive and neglectful parents.

Some studies have shown a correlation between low DβH levels and certain personality traits such as the extroversion scores on the Eysenck Personality Questionnaire [Roy and Brockington, 1987] and sensation seeking [Ballenger et al., 1983; Umberkoman-Wita et al., 1981], while in one study there was a positive association between plasma DβH and sensation seeking scores [Folstein and Rutter, 1977].

In a study of CSF and serum DβH, Major et al. [1980] examined 32 patients from an alcohol rehabilitation unit. Of these, 11 had a secondary diagnosis of personality disorder (two schizoid, four obsessive-compulsive, two asthenic, one passive-aggressive, and two inadequate using DSM II criteria). There was a significant correlation ($r = .6, P = <.01$ between the CSF and serum DβH levels. There was a significant correlation between the CSF DβH levels and the Minnesota Multiphasic Personality Inventory (MMPI) scores of hypochondriasis, depression, hysteria, psychopathic deviation, paranoia, psychasthenia, and social introversion, but no correlation with serum DβH levels. Of the 11 individuals with personality disorder, 10 were in the low CSF DβH group ($P < .001$). The MMPI in the low DβH group meet criteria for the 8-2-4 profile [Works et al., 1974] described as having in common a general distrust of people, a suspicious questioning of others motives, and a fear of emotional involvement with others, in spite of an exaggerated need for attention [Works et al., 1974]. This profile is common in paranoid schizophrenia and antisocial personality disorder. The significant findings with CSF but not serum DβH suggest the serum DβH levels are more susceptible to environmental or other factors, independent of the basic genetic DβH levels, and suggest that short of examining the expression of the DβH levels in the CNS, an examination of DβH alleles might be the most precise approach to examining the potential role of DβH in human behavioral disorders. The same group also examined CSF DβH levels in patients with a variety of psychiatric dis-

orders including major depression, bipolar affective disorder, schizophrenia, and alcoholism [Lerner et al., 1978]. The only significant correlation was between low CSF DβH and bipolar disorder.

In a study of normal college students, Buchsbaum et al. [1978] found a significant association between increased selective attention, as inferred from cortical average evoked response and increased vigilance, as measured by a continuous performance task, with high plasma DβH, especially in subjects with low plasma MAO levels.

A potential role of DβH in learning is suggested by animal studies using DβH inhibitors. In one such study [Isquierdo et al., 1979] the administration of a DβH inhibitor 2 hours prior to training resulted in a 51% decrease in brain norepinephrine and an associated decrease in learning passive and active avoidance. In another, study injection of the DβH inhibitor resulted in amnesia for a spatial discrimination test suggesting norepinephrine depletion impaired a mechanism necessary for memory retrieval [Botwinick et al., 1977]. DβH inhibitors also suppressed the normal enlargement of the locus of the memory trace [Flexner and Flexner, 1976; Flexner et al., 1983].

Since DβH is located in the sympathetic nerve terminals and released into the circulation during the release of norepinephrine, the genes involved in its control could reside at loci other than the DβH gene itself. Thus, association studies between genetic markers at the DβH locus and ADHD and CD could be negative. On the other hand, if the serum levels of DβH are confounded by a range of environmental factors, association studies with genetic markers of DβH could provide a more accurate assessment of the role of the DβH gene in these disorders than blood levels. Linkage studies between the DβH locus and schizophrenia [Aschauer and Meszaros, 1994], alcoholism, depression, manic-depression, and Tourette syndrome [Comings et al., 1986] have been negative. However, some sib pair analyses suggest a weak linkage between the ABO blood group and thus DβH, and some psychiatric disorders such as depression and alcoholism [Wilson et al., 1992].

Since all of the behaviors associated with the inhibition of DβH activity are common in patients with Tourette syndrome (TS) [Comings and Comings, 1984, 1987a; Knell and Comings, 1993; Comings, 1990a] we sought to determine if there was any association between the DβH *Taq* B polymorphism [d'Amato et al., 1989] and TS, conduct disorder, attention deficit hyperactivity disorder (ADHD), autism, or related behaviors. In addition the possible association between a number of individual behavioral clusters and the DβH B1 allele was examined.

Dopamine Transporter

The dopamine transporter directs the re-uptake of dopamine from the synapse back into the presynaptic neuron from which it was released. Since one of the modes of action of cocaine is to inhibit dopamine transporter function [Ritz et al., 1990, 1992], it has been implicated in the biology of drug addiction, as well as other disorders including Parkinson's disease [Uhl, 1990] and Tourette syndrome [Singer et al., 1991]. The human dopamine transporter gene (DAT1) was cloned and sequenced by Vandenbergh et al. [1992b] and has been mapped to chromosome 5p15.3 [Vandenbergh et al., 1992a,b]. The presence of a polymorphic 40 base pair repeat in the 3′ untranslated region of the gene with repeat numbers ranging from 3 to 11 [Vandenbergh et al., 1992a,b], and *Taq* I RFLPs [Vandenbergh et al., 1992b], have allowed an examination of the potential role of the DAT1 gene in a variety of conditions. The 10 and 9 repeats were found to be the most common, accounting for over 90% of the alleles [Vandenbergh et al., 1992a]. Methylphenidate, commonly used in the treatment of ADHD, provides dopaminergic stimulation by inhibition of the dopamine transporter [Schweri et al., 1992] and methylphenidate decreases cocaine craving in cocaine addicts [Khanatzian et al., 1984]. We have used the 40 bp repeat alleles to examine the possible role of the DAT1 gene in a number of different psychiatric disorders and a wide range of TS associated behaviors.

Simultaneous Studies of All Three Dopaminergic Genes

We have also explored the potential of adding an additional feature to maximize the power of association studies to explore polygenic inheritance—the examination of the effect of combining two or more genes on the phenotype, in this case the *Taq* A1 allele of the DRD2 gene, the *Taq* B1 allele of the DβH gene, and the 10/10 genotype of the DAT1 gene.

The concept of psychiatric disorders being polygenic is not new. Plomin has often proposed that this is the most likely mechanism of inheritance for human behavioral disorders [Plomin, 1990; Plomin et al., 1994]. Gottesman [Gottesman, 1991; O'Rourke et al., 1982] and more recently Cloninger have argued that schizophrenia "is caused by the nonlinear interaction of multiple genetic and environmental factors influencing brain development and function" [Cloninger, 1994]. In our 1984 study of the inheritance of Tourette syndrome [Comings et al., 1984], based on an analysis of 250 consecutive pedigrees using the program POINTER, we concluded that TS was due to a single major autosomal dominant gene *only* if the lifetime risk of the disorder was less than 12 per 1,000. Otherwise it was probably multifactorial, polygenic. Our subsequent epidemiological studies of the frequency of TS in school age boys showed a frequency of full TS of 1 in 90, and of probable TS of 1 in 40 [Comings et al., 1990]. More recent studies by Kurlan et al. [1994b], also of school children, showed comparable frequency of TS, and in a recent epidemiological study of Israeli soldiers [Zohar et al., 1992], the frequency of TS or chronic motor tics was 2.6%. Thus, the high frequency of TS is consistent with a polygenic model by segregation analysis. In our more recent pedigree studies, when the entire spectrum of TS related behaviors [Comings, 1990a, 1995e] is included, many families show evidence that the genes are inherited from both parents [Comings et al., 1989; Comings and Comings, 1992]. Kurlan et al. [1994a] have recently verified this.

In the present study the additive effect of these three major genes affecting dopaminergic neurons was studied by determining if subjects who had inherited specific markers of all three genes tended to have higher (worse) behavioral scores than those who inherited less than three. The subtractive effect, the reciprocal of the addictive effect, was examined by determining if those who inherited none of these markers, tended to have lower (better) behavioral scores. Both effects were examined by determining if those who inherited one or two of the markers tended to have intermediate scores. This tests for the essence of polygenic inheritance—the requirement for the additive effect of several genes to produce a clinically significant effect on the phenotype.

METHODS

Subjects

The subjects were patients, or relatives of patients, treated at the TS clinic of the City of Hope National Medical Center. The diagnoses of TS, chronic motor tic disorder or chronic vocal tic disorder, was based on the DSM-III-R [1987] criteria. The TS probands (n = 225) are defined as the individuals who sought medical care at this clinic. Of the probands, 82% had TS while the remaining 18% had either chronic motor tic disorder or chronic vocal tic disorder. Among the non-proband TS relatives (n = 60), 54% had TS, 31% had chronic motor tic disorder, and 15% chronic vocal tic disorder. None of the non-TS relatives (N = 132) had chronic motor or vocal tics. All probands were personally interviewed and examined. Over 80% of the relatives were also personally interviewed. Each proband and their relatives were questioned about the racial and ethnic background for their four grandparents. For the DRD2 studies only non-Hispanic Caucasian probands, relatives and controls with northern and western European background were included. All subjects were over 6 years of age. All subjects or families signed informed consents and the studies were approved by the Institutional Review Board.

Controls

The controls (n = 67) came from three sources: a) adopting, foster, or step parents of TS patients; b) subjects from an endocrinology clinic with thyroid cancer or non-insulin dependent diabetes mellitus; and c) hospital personnel including professionals, technicians, and maintenance workers. The endocrine patients were chosen as controls because both conditions are readily treatable with a high cure rate and produce a minimal disruption of daily living, present at a wide range of ages, and the patient base was the same at that for the TS subjects. All controls were screened to exclude alcohol, drug, and tobacco abuse.

Structured Questionnaire

Since 1987 all patients and available first degree relatives were required to fill out a detailed 31 page behavioral questionnaire modeled after the Diagnostic Interview Schedule (DIS) [Robins et al., 1981]. The complete questionnaire is available elsewhere [Comings, 1990a]. In addition to the DIS questions, there were many questions concerning demographic variables, all of the DSM-III and DSM-III-R [1980, 1987] variables required for the diagnosis of ADHD, and questions about the type, location, duration and severity of motor and vocal tics. The responses to these questions were then entered into an SPSS data base. Various aspects of the use of this instrument and the use of symptom clusters are presented elsewhere [Comings, 1994a,b, 1995a,d].

Blood Samples

While blood samples were not obtained on every TS proband or relative attending the clinic, the selection was essentially random within the confines of the following considerations. First, providing blood samples was totally voluntary and many subjects, especially the younger ones, chose not to have blood drawn. Secondly, because of the concerns about racial and ethnic stratification, there was a tendency to draw blood from non-Hispanic, northern and western European Caucasians since the most extensive data on controls comes from this group. Third, if both parents were available, there was a preference for obtaining blood from these families versus families where one of the parents was unavailable.

Genotyping

All subjects were genotyped based on a neutral identification number and read without knowledge of the individual being typed.

DRD2 polymorphism. The D_2A1 genotyping was performed by hybridization of Southern blots as described previously [Comings et al., 1991]. Some were also genotyped by a PCR technique.

DβH polymorphism. d'Amato et al. [1989] reported the presence of two *Taq* DβH polymorphisms entitled A and B. A DβH cDNA clone A11 [Lamouroux et al., 1987, 1993] was used consisting of a 2.7 kb insert at the EcoR1 site. To improve labeling the vector was digested with *Bam* H1 and *Sal* I to produce five bands. A 3.5 kb fragment was labeled for testing the B polymorphism. Digestion with *Taq* I restriction endonuclease, electrophoresis in agarose, Southern transfer to a nylon filter, hybridization with ^{32}P labeled probe, and autoradiography demonstrated fragments of 2.8 kb (B1), and 1.4 kb (B2).

Determination of the DAT1 repeat polymorphism. The alleles at the 3′ UTR were determined by PCR using the oligomers and PCR conditions reported by Vandenbergh et al. [1992b]. Following PCR amplification the products were electrophoresed in an 8% acrylamide gel with a set of size markers.

Criteria for Comorbid Conditions

The structured questionnaire allowed us to examine sets of related symptoms. The criteria for each of these was determined prior to the present study and many have already been published or are in press as follows: alcohol abuse [Comings, 1994c], drug abuse [Comings, 1994b], obsessive-compulsive behaviors, major depressive episode, mania, somatization disorder, panic attacks, phobias, conduct disorder, oppositional-defiant disor-

der (ODD), sexual disorders learning disorders, and tic severity [Comings, 1994a, 1995a,d,e]. The ADHD score represented the sum of all 22 of the ADHD variables used in the questionnaire where no or never = 0, occasionally = 1, and often = 2. Based on prior studies [Knell and Comings, 1993] those with a score of 21 or greater were considered to have ADHD. Because of our particular interest in ADHD, we also examined it using a second set of criteria based strictly on the DSM-III-R [1987] diagnosis where at least 8 out of 14 of the above 22 variables were required.

The accuracy, utility and sensitivity of a questionnaire based approach to symptom evaluation has been demonstrated by others [Gadow and Sprafkin, 1994; Grayson and Carlson, 1991] by comparing the use of such an instrument to an interviewer administered structured instrument such as the Kiddie Schedule for Affective Disorders and Schizophrenia, both given to the same subjects.

The following are the criteria for some of the symptoms or scores that were not published at the time of this writing or may require clarification.

Grade School

To assess general performance in school, we asked the following question: "For grades 1 to 6 was your school performance on the whole below average, average, or above average in the following: a) math, b) reading, and c) writing?" The possible answers were below average (2), average (1), and above average (0). The results were summed across the three subjects with the worst score being 6 and the best 0. For the dichotomous variable a score of 4 or more was considered positive for grade school problems.

Learning Disabilities

Individuals were considered to have had problems with learning disabilities if they ever had to be placed in an educationally handicapped, learning handicapped, learning disorder, or resource class.

Phobias

Subjects were asked about the presence of all of the 16 phobias in the DIS and were considered to have problems with phobias if they had difficulty with 3 or more. The phobia score was the total number of DIS listed phobias for a given subject.

Panic Attacks

Panic attacks were considered to have been present at some time if individuals answered yes to the DIS question. "Have you ever had a spell or attack (not due to a physical illness) when all of a sudden you felt frightened, anxious, or very uneasy where most people would not be afraid?" The panic score represented the total number of DIS panic attacks symptoms for a given subject.

Reading Problems

To evaluate reading problems individuals were asked, "What is the greatest number of years you were felt to be behind your peers in reading, if any? For example, if when you were in the 6th grade and you were only reading at 4th grade level, you would have been 2 years behind." Those who answered 2 years or more were considered to have had reading problems.

Stuttering

This was evaluated by the question, "Have you ever had problems with stuttering?"

General Anxiety

To have been scored positive for general anxiety individuals had to answer yes to both of the following DIS questions, "Have you ever had period of excessive anxiety or worry about various things in your life?" and "If yes, have these feelings persisted for a period of 6 months or more when they were present more than they were absent?"

Somatization

Problems with somatization were considered to be present if the individuals answered yes to three or more of the DIS questions concerning somatization. The somatization score represented the total number of DIS somatization symptoms for a given subject.

Sleep

Sleep problems were considered to be present if any one of the following were present daily or almost daily: problems getting to sleep at night, sleep walking, night terrors, early wakening and unable to get back to sleep, sleep talking, or nightmares. The sleep score represented the total number of the above sleep score symptoms that occurred daily or almost daily.

Sexual

Individuals were scored positive if any of the following were present [Comings, 1994a]: 1) frequent public exhibitionism, 2) sex drive much greater than average, 3) prefer the same sex or both sexes, 4) a precocious interest in sexual things, 5) as a child drew dirty pictures much more than other children of their age, 6) persistently felt they were born to the wrong sex, 7) dressed as the opposite sex other than for Halloween or a costume party, 8–11) a period of 6 months or more of being sexually aroused by objects, or children, or masochistic or sadistic fantasies. The sexual behavior score represented the total number of the above sexual behavior problems in a given subject.

Schizoid Behaviors

Subjects were scored positive if they answered yes to two or more of the DIS questions on schizophrenic symptoms. The schizoid score represented the total number of DIS schizoid symptoms in a given subject.

Tics

Questions were asked about the presence and age of onset of eight different motor tics and nine different vocal tics. The number of such tics was added up to produce a tic score. Thus, the presence of multiple different tics was scored higher than few tics.

The above provided dichotomized results for chi square analysis, and scores for comparing the means in specific allele carriers versus non-carriers.

Grouping of Cases

The above information allowed us to place subjects into two different types of groupings. The first was based on the presence or absence of chronic motor and/or vocal tics. These groups were TS probands, relatives of TS probands with TS or chronic tics, relatives of TS probands without chronic tics, and controls without tics. A second grouping consisted of a) controls without drug, alcohol or tobacco abuse and without the behavioral condition in question (*controls without*), b) TS probands or their relatives without the behavioral condition in question (*cases without*), and c) TS probands or their relatives with the behavioral condition in question (*cases with*). Thus, for example, since the controls as well as the subjects completed the structured questionnaire, for obsessive compulsive behaviors the comparison would be between controls without substance abuse and without obsessive compulsive behaviors, vs. TS probands and relatives without obsessive compulsive behaviors vs. TS probands and relatives with obsessive compulsive behaviors. Of the TS probands in the *polygenic set* only 3% were mild, 74% were moderate, and 23% were severe.

Statistics

When examining individual behaviors, such as behavior x, there would be two possible reasons for a significant increase in the prevalence of a marker in TS probands with behavior x vs. controls without behavior x: a) the marked gene may play a role in behavior x, or b) the marked gene may be increased in frequency because it is associated with behavior y, which itself is associated with behavior x. To distinguish between a) and b) we required that there be a significant, progressive, linear increase in the prevalence of the marker across all three groups, *controls without* (without x and very likely without y), *cases without* (without x but often with y), and *cases with* (with x and often with y). The SPSS (SPSS, Inc., Chicago) statistical packages were used. When comparing dichotomous variables for a progressive series of *controls without* vs. *cases without* versus *cases with,* the SPSS Mantel-Haenzel chi square statistic for a progressive linear trend was used.

For comparison of the means of a given behavioral score in subjects with the D_2A1 allele (D_2A1A1 or D_2A1A2) versus subjects without the A1 allele (D_2A2A2) the Student t-test was used.

For uniformity for each of the genes studied we genotyped the same group of subjects for three different genes. This group was termed the *polygenic set.* Subjects were placed into this set before they were genotyped for DβH or DAT1 alleles. The selection criteria for the polygenic set was simple: subjects had to be non-Hispanic Caucasians, had to have filled out the structured questionnaire, had to have agreed to have blood drawn, had to have produced a sufficient amount of DNA to genotype all three genes, and had to be either a control, a TS proband or the relative of a proband. Since the same subjects were tested, this allowed the means for the behavioral scores to be compared for the different genes. There are 319 subjects in the *polygenic set.* In addition, a significant number of subjects were genotyped for one of the genes, but not for all three. For each gene, this was termed the *total set* and varied in size for the different genes. In every case the *total set* included the *polygenic set* plus additional non-polygenic set cases. To avoid losing data or power, for each behavior, the *total set* was also examined. However, to save space, only the *P* values for the *total set* are given in the last column of the appropriate tables. The t-test analyses compared the means of the different behavioral variables for subjects with the allele or marker being tested versus those without the allele or marker. Again, where appropriate, the p value for the *total set* is given in the final column of the respective tables.

For the quantitative tests of the means of the different behavioral scores, we envisioned two somewhat opposing strategies. The first was to examine as large a number of subjects as possible, on the assumption that the greater the number of subjects examined the less the chance for a type II error. For this strategy controls, relatives and TS probands were examined. The second strategy was to only compare the extremes, i.e., controls versus TS probands, on the assumption that this would compare individuals with the least versus the greatest degree of expression of the mutant genes. The results will be presented for the first strategy, and the results of the second will be discussed if they are more informative than examining the larger set. Bonferroni adjusted α values are given in the appropriate tables, i.e., for multiple comparisons of subjects in mutually exclusive categories.

ANOVA analysis was performed using linear contrast [Dunn and Clark, 1995] to determine if there was a significant linear decrease in the means of a number of continuous behaviors across the four groups where 3 of 3, 2 of 3, 1 of 3, and 0 of 3 markers were present. The Tukey test was incorporated into the analysis to determine if any of the individual group scores were significantly different from each other, at $\alpha = 0.05$.

To estimate the percent of the variance due to the three dopaminergic genes, multiple linear regression analysis was performed using 1 as the presence of the marker and 0 as the absence of the marker versus the different behavioral scores, for all three genes simultaneously. R^2 gave proportion of the variance due to each gene and the sum of r^2 for all three genes. This provides the total proportion of the variance accounted for by all three genes together, for each specific behavior.

RESULTS

Age and Sex

The number, mean age, and sex distribution of the subjects in the four groups of subjects for the total set, are shown in Table I. It was expected that the mean age of the TS probands would be less than for the other groups and this was the case. It was also expected that the M:F sex ratio of the TS probands and relatives with TS would be higher than in the non-TS groups and this was also the case. However, since the genes tested are

TABLE I. Age and Sex of the Different Subject Groups

Group	N	Mean age	S.D.	
Age				
TS probands	225	16.83	12.11	
Relatives with TS	60	27.72	14.73	
Relatives without TS	132	38.07	11.95	
Controls	67	42.31	14.86	
Total	484			
	N males	N females	N total	% males
Sex				
TS probands	188	37	225	84
Relatives with TS	37	23	60	62
Relatives without TS	51	81	132	39
Controls	27	40	67	40
Total			484	

all autosomal, the sex ratio itself would not be expected to be a factor. To test this we performed chi square analyses on the presence or absence of the different markers vs. sex. These were not significant.

Taq A1 Allele of the DRD2 Gene

Controls without* vs. *cases without* vs. *cases with. The prevalence of the D_2A1 allele in the four groups of TS probands, TS relatives with chronic tics, TS relatives without chronic tics, and controls, for the *polygenic set* and *total* set are shown in Table II. In both cases there was a significant progressive increase by Mantel-Haenszel linear chi square, in the prevalence of the A1 allele from controls (23.5 and 26.9%) to TS probands (41.5 and 41.7%).

The results of determining the prevalence of the D_2A1 allele in *controls without,* vs. *cases without* vs. *cases with,* for the *polygenic set,* and the *P* values for the significant associations for the *total set* are shown in Table III. The most significant association was with manic symptoms, where 21.2% of controls who never had manic symptoms carried the D_2A1 allele, compared to 28.7% of cases without manic symptoms, vs. 52.2% of the cases with symptoms ($P = .00024$). The other significant variables, in order, were oppositional defiant, sexual, ADHD-R, schizoid, ADHD, tics, major depression, and conduct. The most significant association for the *total set* was with sexual ($P = .0007$), stuttering ($P = .0008$), schizoid ($P = .0016$), and mania ($P = .0017$).

T-statistic for means for D_2A1 carriers* vs. *D_2A2A2 carriers. Since the majority of the behavioral assessments involved a continuous score, it was also useful to examine the means of these scores for all subjects based on whether they carried the D_2A1 allele (D_2A1A1 and D_2A1A2) or not (D_2A2A2). These results for subjects in the *polygenic set* are shown in Table IVA listed by decreasing t-test value. The *P* values for the significant behaviors in the *total set* are shown in the last column. The significant variables, in order, were ADHD, mania, ADHD-R, conduct, tics, oppositional defiant, schizoid, and sexual. For the *total set* three additional variables, stuttering, obsessive-compulsive, and somatization were also significant. Most of these remained significant when the controls were deleted (Table IVB).

For comparison with the results used for all three dopaminergic genes together, the significant results for controls and TS probands for the *polygenic* are listed in Table IVC. Here conduct ranked the highest, followed in order by mania, ADHD, tics, schizoid, obsessive-compulsive, and oppositional defiant.

To determine if homozygosity for the D_2A1 allele (D_2A1/D_2A1) gave higher mean behavior scores than heterozygotes (D_2A1/D_2A2) the means for these two groups for all the behavioral scores were examined. For every variable except the tic score, the mean was lower for the homozygotes than the heterozygotes. In only three variables was this significant: alcohol, grade school, and read.

Taq B1 Allele of the DβH Gene

The* Taq *B1 allele in various psychiatric disorders. Of 148 non-Hispanic Caucasian controls tested, 60.8% carried the DβH B1 allele. Of those screened to exclude alcohol, drug and tobacco abuse, or dependence, 52.9% carried the B1 allele (Table V). Using an α of 0.05, there was a significant increase in prevalence of the B1 allele to 70.5% in 352 TS probands ($P = .012$). To determine if severity of TS played a role, the TS probands were divided by a global rating into mild (too mild to require treatment of any aspect of the TS spectrum), moderate (some aspect requiring treatment), and severe (some aspect of the TS spectrum causing a major disruption in their life [Comings and Comings, 1985]). There was an increase in prevalence of the B1

TABLE II. Prevalence of the D_2A1 Allele in the Various Subject Categories

Category	N	A1A1	A1A2	A2A2	%A1	Freq.	χ^{2a}	*P*
A. Polygenic set (n = 319)								
TS probands	142	9	50	83	41.5	.24		
Relatives with TS	39	0	11	28	28.2	.14		
Relatives without TS	104	4	24	76	26.9	.15		
Controls	34	1	7	26	23.5	.14	6.99	.0082
B. Total set (n = 484)								
TS probands	225	13	81	131	41.7	.24		
Relatives with TS	60	2	19	39	35.0	.19		
Relatives without TS	132	4	34	94	28.8	.16		
Controls	67	3	15	49	26.9	.16	8.35	.0038

[a]Mantel-Haenzel linear chi square based on D_2A1 prevalence.

TABLE III. Association of the D_2A1 Allele With Various Comorbid Behaviors in the Groups *Controls Without*† *Cases Without* and *Cases With* the Behavior, for the Polygenic Set

	Controls without		*Cases without*		*Cases with*				
Score	N	%	N	%	N	%	χ^{2*}	*P**	*P* for total set*
Polygenic set									
Manic	33	21.2	216	28.7	69	52.2	13.47	.00024	.0017
Oppositional defiant	34	23.5	206	29.6	79	46.8	8.31	.0039	.0274
Sexual	27	25.9	204	28.4	88	46.6	8.19	.0042	.0007
ADHD-R	34	23.5	210	30.5	75	45.3	6.63	.010	.0085
Schizoid	32	25.0	194	30.4	88	44.3	5.92	.015	.0016
ADHD	34	23.5	163	30.1	116	41.4	5.42	.020	.0110
Tics	34	23.5	104	26.9	181	38.7	5.27	.022	.0088
Major depression	25	24.0	176	30.1	109	41.3	4.70	.030	N.S.
Conduct	26	23.1	161	30.4	124	39.5	3.93	.047	.0135
Obsessive-compulsive	32	21.9	216	32.4	69	40.6	3.59	.059	.0013
Reading	18	16.7	116	31.0	169	36.7	3.01	.082	N.S.
Learning	29	20.7	190	32.6	95	37.9	2.70	.100	.041
Sleep	28	21.4	202	32.7	83	38.6	2.64	.103	.049
Panic attacks	28	21.4	178	32.6	100	38.0	2.58	.107	N.S.
Stuttering	33	21.2	241	32.8	41	39.0	2.53	.111	.0008
Alcohol abuse	34	23.5	262	33.6	23	43.5	2.53	.111	.037
Phobias	23	17.4	182	33.0	103	36.9	2.40	.120	N.S.
Drug abuse	34	23.5	258	33.7	27	40.7	2.09	.147	N.S.
Somatization	19	26.3	154	32.5	78	39.7	1.78	.182	N.S.
General anxiety	26	15.4	226	35.4	67	32.8	0.98	.322	N.S.
Grade school	30	23.3	187	34.2	88	34.1	0.60	.436	N.S.

†Controls without, controls without alcohol or drug or tobacco abuse/dependence and without the behavior in question. Cases without, TS probands and TS relatives that did not have the behavior in question. Cases with, TS probands and TS relatives that did have the behavior in question.
*Mantel-Haenszel linear chi square or *P* value based on the Mantel-Haenszel linear chi square.

allele from 54.3% for mild, to 72.1% for moderate, to 72.7% for severe. The prevalence of the B1 allele for the moderate cases was significant at $P = .0071$.

The prevalence of the B1 allele was 73.1% for 78 subjects with ADHD ($P = .019$) and 73.1% for 104 smokers ($P = .012$). The prevalence of the B1 allele in the other groups was not significantly increased over that in controls. While the association of the B1 allele with TS and smoking was significant only without a Bonferroni correction, there is some concern that such a correction may inappropriately increase type II errors [Rothman, 1990].

In a post hoc analysis of B1/B1 homozygosity versus B1/B2 heterozygosity in the three grades of TS, it was noticed that 37.1% of mild, 49% of moderate, and 62% of severe TS cases were B1/B2 heterozygotes (Mantel-Haenszel linear chi square = 6.25, $P = .012$).

Controls without, cases without, cases with. The results for the prevalence of the DβH *Taq* B1 allele for 319 subjects in the *polygenic set* are shown in Table VI. ADHD was the most significant with a B1 allele prevalence of 47.1%, for the controls without substance abuse or ADHD, 70.6% for the cases without ADHD, and 81.9% of cases with ADHD ($P = .0001$). Other significant behavioral variables, in order, were learn, grade school, ADHD-R, oppositional defiant, tics, mania, alcohol, reading, drug abuse, sleep, stuttering, and obsessive-compulsive. The results for the *total set* (not shown) were similar with ADHD again the most significant. When males only were examined in the *total set* sleep was most significant ($P = .00005$), then ODD ($P = .002$), and ADHD ($P = .005$).

T-statistic for means for B1 carriers* vs. *B2B2 carriers. Table VII shows the results when the *polygenic set* was restricted to controls and TS probands. ADHD was again at the top of the list and was significant at $P = .020$. The only other significant behavior was grade school, $P = .029$. The significant results for the *total set* are listed in Table VIIB. Oppositional defiant behavior, sleep, ADHD and read were significant at $\alpha = 0.05$.

To determine if homozygosity for the B1 allele (B1/B1) gave higher mean behavior scores than heterozygotes (B1/B2) the means for these two groups for all the behavioral scores were examined. There were no significant differences for any of the behavioral variables for homozygotes versus heterozygotes.

10/10 Genotype of the DAT1 Gene

The frequency of the different DAT1 alleles for the entire set of subjects examined is shown in Table VIII. To simplify the analyses the prevalence of the 10/10 genotype was compared to the prevalence of the 10/x or x/x genotypes for Tourette syndrome and autism, and different categories of behavioral disorders. Of the 91 controls, 37.4% carried the 10/10 genotype. This increased to 52.3% for 241 TS probands ($P = .015$). There was no significant difference in the mild, moderate, and severe TS subjects. Among 36 subjects with autism 58.3% were 10/10 ($P = .031$). The results for TS probands and TS relatives were still significant after a Bonferroni correction. Examination of the frequency of the 10 allele gave comparable results (last two columns in Table VIII).

TABLE IV. Comparison of Mean Behavior Scores for D_2A1 Carriers vs. D_2A2A2 Carriers

	D2A1		D2A2A2				
Score	Mean	S.D.	Mean	S.D.	t	*P*	*P* for total set*
A. *Polygenic set,* controls, TS relatives and TS probands (N = 319, D_2A1 = 106, D_2A2A2 = 213)							
ADHD	20.77	13.22	15.53	13.60	3.28	.001	.003
Mania	2.00	2.49	1.16	1.83	3.19	.002	.003
ADHD-R	4.97	4.42	3.40	4.30	3.02	.003	.012
Conduct	3.01	2.39	2.24	1.99	2.87	.005	N.S.
Tics	3.05	3.74	1.92	3.00	2.70	.008	.042
Oppositional defiant	3.11	3.22	2.22	2.79	2.44	.016	N.S.
Schizoid	1.66	2.19	1.11	1.34	2.41	.017	.007
Sexual	0.78	1.33	0.46	0.96	2.19	.030	.017
Obsessive-compulsive	2.90	3.05	2.28	2.64	1.79	.074	.009
Drugs	0.59	1.68	0.34	1.24	1.34	.181	N.S.
Somatization	2.62	3.31	2.11	2.87	1.24	.217	.048
Learn	0.57	0.89	0.45	0.81	1.17	.245	N.S.
Major depression	3.76	3.09	3.35	3.01	1.13	.260	N.S.
Stuttering	0.16	0.37	0.11	0.32	1.11	.268	.002
Phobia	2.61	2.82	2.28	2.74	1.00	.319	N.S.
Panic	3.17	2.20	2.94	2.07	0.92	.360	N.S.
Read	1.78	1.93	1.57	1.99	0.91	.365	N.S.
Sleep	0.47	0.79	0.39	0.79	0.77	.443	N.S.
Grade school	2.75	1.93	2.57	1.95	0.75	.457	N.S.
Alcohol	0.60	2.27	0.41	1.93	0.72	.471	N.S.
General anxiety	0.23	0.43	0.21	0.41	0.40	.692	N.S.
B. *Total set,* TS relatives and TS probands only (N = 417, D_2A1 = 153, D_2A2A2 = 264)							
Mania	2.03	2.48	1.37	1.94	2.83	.005	
Conduct	3.15	2.36	2.50	2.13	2.82	.005	
Schizoid	1.81	2.24	1.22	1.74	2.80	.006	
Stuttering	0.24	0.43	0.13	0.34	2.69	.008	
ADHD	22.26	12.87	18.71	12.94	2.60	.010	
Obsessive-compulsive	3.24	3.11	2.48	1.69	2.51	.013	
Somatization	2.93	3.51	2.01	2.70	2.49	.014	
Sexual	0.80	1.29	0.50	1.00	2.44	.015	
ADHD-R	5.28	4.46	4.36	4.62	1.99	.048	
Tics	3.11	3.54	2.52	3.32	1.67	.096	
C. *Polygenic set,* controls and TS probands only							
Conduct	3.65	2.47	2.64	2.10	2.80	.006	
Mania	2.31	2.50	1.40	1.95	2.54	.012	
ADHD	25.57	12.16	21.23	14.53	2.12	.036	
Tics	4.25	3.88	3.06	3.44	2.06	.042	
Schizoid	1.91	2.58	1.20	1.53	2.02	.046	
Obsessive-compulsive	3.40	3.24	2.44	2.80	2.01	.047	
Oppositional defiant	4.31	3.27	3.30	3.17	2.01	.046	

*Total set: N = 417, D_2A1 = 153, D_2A2A2 = 264.

TABLE V. Prevalence of the DβH *Taq* B Alleles in Various Psychiatric Disorders

Disorder	N	11	12	22	%1	O.R.	freq1	χ^a	*P*
Controls	148	21	69	58	60.8		.37		
Screened cont.	51	6	21	24	52.9		.32		
TS	352	71	177	104	70.5	1.54	.45	6.29	.012
Mild	35	6	13	16	54.3	0.76	.36	0.014	.903
Moderate	251	58	123	70	72.1	1.67	.48	7.24	.0071
Severe	66	7	41	18	72.7	1.72	.42	4.82	.027
ADHD	78	18	39	21	73.1	1.75	.48	5.46	.019
Alcoholism	23	3	13	7	69.6	1.47	.41	1.77	.183
Autism	40	6	21	13	67.5	1.34	.41	1.95	.163
Depression	28	6	8	14	50.0	0.64	.36	0.06	.803
Drug abuse	29	2	17	10	65.5	1.22	.36	1.18	.277
Gamblers	111	11	56	44	60.4	0.98	.35	0.78	.375
Smokers	104	29	47	28	73.1	1.75	.51	6.18	.012
Total	913								

[a]Comparison of the prevalence of the B1 allele in screened controls (no alcohol, drug or tobacco abuse/dependence) versus the disorders in question. O.R., odds ratio. Bonferroni corrected α = .05/8 = .0065.

TABLE VI. DβH *Taq* B1 Allele (11 + 12 Genotype) in Controls, TS Probands, and Relatives, for the *Polygenic Set*

	Controls without		*Cases without*		*Cases with*			
Score	N	%	N	%	N	%	χ^{2a}	*P*
ADHD	34	47.1	163	70.6	116	81.9	15.14	.00010
Learn	29	48.3	190	72.6	95	81.1	10.13	.0014
Grade school	30	46.7	187	72.2	88	79.5	9.48	.0021
ADHD-R	34	47.1	210	74.3	75	78.7	8.52	.0035
Oppositionally defiant	34	47.1	206	74.8	79	77.2	7.21	.0070
Tics	34	47.1	104	75.0	181	75.7	7.17	.0074
Mania	33	48.5	216	75.0	69	76.8	6.02	.014
Alcohol	34	47.1	262	76.0	23	69.6	5.92	.018
Reading	18	44.4	116	73.3	169	76.9	5.42	.019
Drug abuse	34	47.1	258	76.0	27	70.4	5.33	.021
Sleep	28	53.6	202	74.3	83	78.3	4.63	.031
Stutter	33	48.5	241	76.3	41	73.2	4.56	.032
Obsessive compulsive	32	46.9	216	75.9	69	73.9	4.33	.037
Schizoid	32	50.0	194	76.8	88	73.9	2.94	.086
Somatization	19	47.4	154	76.0	78	75.6	2.62	.105
Panic attacks	28	50.0	178	76.4	100	74.0	2.43	.119
Major depression	25	52.0	176	75.6	109	75.2	2.40	.121
Conduct	26	50.0	161	77.0	124	73.4	1.61	.203
Sexual	27	55.6	204	74.5	88	72.7	1.12	.288
Phobias	23	56.5	182	76.4	103	73.8	0.70	.401
General anxiety	26	53.8	226	75.2	67	70.1	0.62	.430

[a]See Table III.

Controls without, cases without, and cases with. These results for the *polygenic set* of 319 subjects, are shown in Table IX. The variable with the highest chi square was somatization with 21.1% of the controls without somatization problems carrying the 10/10 genotype, vs. 46.8% of the TS probands or relatives without somatization problems, vs. 60.3% of TS probands or relatives with somatization problems ($P = .0009$). The other significant variables, in order of the magnitude of the chi square, were alcohol, ADHD-R, major depression, panic, obsessive compulsive, general anxiety, and mania. The significant results for the modestly larger *total set* of 357 subjects, are shown in the last column of Table IX. The major difference was the addition of the oppositional defiant, sexual, read, and ADHD as significant variables.

T-statistic for means for 10/10 genotype* vs. *the non-10/10 genotype. When the *total set* was examined, the variables somatization and major depression showed significantly higher means for those with the 10/10 genotype (Table XA). None of the variables were significant for all subjects of the *polygenic set.* The results for the controls and TS probands only, for the *polygenic set,* are given in Table XB. This is the set used in the examination of the additive effects of the three. Here the following variables, in order, were significant: general anxiety, major depression, ADHD-R, ADHD, and alcohol.

Comparisons of All Three Dopaminergic Genes

The results for each of the behavioral scores studied across all three genes are shown in Table XI. The

TABLE VII. Comparison of Mean Behavior Scores for DβH *Taq* B1 Carriers vs. B2B2 Carriers

	B1		B2B2			
Score	Mean	S.D.	Mean	S.D.	t	*P*
A. *Polygenic set,* controls and TS probands (N = 176, B1 = 124, B2B2 = 52)						
ADHD	24.48	13.57	19.07	12.72	2.37	.020
Grade school	3.26	1.96	2.55	1.88	2.22	.029
B. *Total set,* controls and TS probands (N = 292, B1 = 207, B2B2 = 85)						
Oppositional defiant	4.16	3.19	3.08	3.12	2.68	.008
Sleep	0.63	1.03	0.36	0.70	2.60	.010
ADHD	25.17	13.73	20.86	14.66	2.31	.023
Read	2.14	2.04	1.57	2.09	2.11	.037

TABLE VIII. Prevalence of the Different DAT1 40 bp Repeat Polymorphism Genotypes and Frequencies of the Alleles for Different Groups of Patients†

Subjects	N	6/6	8/9	9/9	8/10	9/10	10/10	9/11	10/11	11/12	%10/10	O.R.	f9	f10	χ^{2*}	P	χ^{2**}	P
Controls	91	1	0	12	0	40	34	1	3	0	37.4		.36	.61				
Tourette syndrome	241	2	1	15	0	92	126	0	4	1	52.3	1.84	.25	.72	5.89	.015	7.93	.0048
Mild	15	0	0	2	0	5	8	0	0	0	55.3	1.92	.25	.74	1.37	N.S.	1.75	.184
Moderate	132	0	1	5	0	56	66	0	3	1	50.0	1.92	.30	.70	3.47	.062	4.27	.039
Severe	94	2	0	8	0	31	52	0	1	0	53.3	2.08	.25	.72	5.99	.014	5.15	.023
TS relatives	103	0	0	7	0	37	57	0	2	0	55.3	2.08	.25	.72	6.27	.012	5.43	.019
Autism	36	0	0	2	1	12	21	0	0	0	58.3	2.35	.22	.75	4.62	.032	4.63	.031
Total	471																	

*Chi square of comparison of prevalence of 10/10 genotype in controls versus the subjects in question.
**Chi square of comparison of the frequency of the 10 allele in controls versus the subjects in question.
†O.R., odds ratio. Bonferroni corrected $\alpha = 0.05/3 = .017$.

groups consisted of those who inherited all three markers (Group 1), 2 of the 3 (Group 2), 1 of the 3 (Group 3), and none of the 3 (Group 4).

The comorbid behavior showing most significant linear association with the four gene groups was ADHD ($P = .0002$). For example, the ADHD score for those who inherited 3 of 3 markers was 30.04, for those who inherited 2 of 3, 24.74, for 1 of 3, 20.42, and 0 of 3, 14.07. The mean for group 4 (none of 3) was significantly less than the mean scores for both group 1 and 2, and the mean for group 3 was significantly less than for group 1. The next most significant was the score for stuttering: 1.17, 1.06, 0.94, and 0.46 ($P = .0002$). The respective scores for oppositional defiant behaviors for groups 1 through 4, were 5.04, 3.91, 3.38, and 1.93 ($P = .0023$). The respective scores for conduct disorder were 4.08, 3.05, 2.87, and 1.93 ($P = .0023$). The other significant variables were tics, obsessive-compulsive, mania, alcohol, and general anxiety. While the remaining behaviors were not significant, 16 of 20 showed the same progressive linear decrease with less genetic loading. The results were similar when the entire *polygenic set* was used, but moderately less significant.

To examine these relationships in further detail, the most significant behavioral score, ADHD was divided into eight groups representing all possible combinations of the markers of the three genes (Table XII). This confirmed the additive and subtractive trend. A similar breakdown of the tic score is also presented. These results were similar to those for the four gene categories and were significant for the same variables.

To examine the possibility that the results might somehow be driven by an unidentified aspect of the controls, the analysis was repeated using only subjects

TABLE IX. Association of the Dopamine Transporter 10/10 Genotype With Various Comorbid Behaviors in Controls and Cases, for *Polygenic Set* (1 = 10/10 Genotype)

	Controls without		*Cases without*		*Cases with*				
Score	N	%1	N	%1	N	%1	χ^{2*}	P*	P* for total set*
Somatization	19	21.1	154	46.8	78	60.3	9.51	.0020	.0009
Alcohol	34	35.3	262	50.4	23	69.6	6.37	.011	.0027
ADHD-R	34	35.3	210	49.5	75	58.7	5.02	.024	.004
Major depression	25	24.0	176	50.0	109	55.0	5.39	.020	.006
Panic	28	28.6	178	50.0	100	55.0	4.53	.033	.010
Obsessive-compulsive	32	31.3	216	50.5	69	56.5	4.59	.032	.010
General anxiety	26	26.9	226	50.9	67	56.7	4.94	.026	.011
Mania	33	33.3	216	50.5	69	56.5	4.07	.044	.012
Oppositional defiant	34	35.3	206	50.5	79	55.7	3.32	.068	N.S.
Sexual	27	33.3	204	50.0	88	55.7	3.41	.064	.013
Read	18	38.9	116	49.1	169	53.8	1.63	.201	.043
ADHD	34	35.3	163	51.5	116	53.4	2.29	.129	.049
Sleep	28	35.7	202	50.5	83	55.4	2.66	.102	N.S.
Stutter	33	33.3	241	52.3	41	51.2	1.96	.164	N.S.
Drug abuse	34	35.3	258	51.9	27	51.9	1.99	.158	N.S.
Learn	29	37.9	190	51.1	95	53.7	1.56	.210	N.S.
Tics	34	35.3	104	55.8	181	49.7	0.49	.482	N.S.
Schizoid	32	37.5	194	52.6	88	51.1	0.78	.370	N.S.
Grade school	30	36.7	187	52.4	88	47.7	0.19	.659	N.S.
Conduct	25	34.6	161	54.0	124	49.2	0.21	.644	N.S.
Phobia	23	21.7	182	54.9	103	46.6	0.40	.525	N.S.

*Mantel-Haenszel linear chi square or P based on the Mantel-Haenszel linear chi square.

TABLE X. Comparison of the Mean Behavioral Scores in Controls and Cases

Score	10/10 Mean	10/10 S.D.	10/x, x/x Mean	10/x, x/x S.D.	t	P
A. *Total set,* controls, TS probands and their relatives (N = 357, 10/10 = 182, 10/x, x/x = 175)						
Somatization	2.69	3.30	1.94	2.68	2.11	.036
Major depression	3.87	3.05	3.20	2.98	2.11	.036
B. *Polygenic set,* controls and TS probands only (N = 176, 10/10 = 85, 10/x, x/x = 91)						
General anxiety	0.33	0.47	0.16	0.37	2.55	.012
Major depression	4.00	3.04	2.87	2.83	2.53	.012
ADHD-R	6.60	4.75	4.91	4.45	2.43	.016
ADHD	25.44	13.94	20.42	13.28	2.43	.016
Alcohol	0.68	2.46	0.09	0.70	2.11	.037

with TS (probands and relatives). Despite the narrowed range of the scores, and the resultant higher P values, ADHD, ADHD-R, somatization, and major depression were significant at $P < .05$, and conduct, oppositional defiant, and mania were marginally significant at $P < .07$. For example, the values for the ADHD score for groups 1 to 4 were 29.9, 26.1, 23.0, and 22.9, respectively ($P = .01$); for conduct score were 5.1, 4.0, 3.8, and 3.2; and for oppositional defiant score were 3.8, 3.3, 2.9, and 2.7.

The results for the estimation of the proportion of the variance for the more significant behavioral variables is shown in Table XIII. In general the DRD2 gene contributed the most to the variance. For the major behaviors associated with TS, between 3.0 and 6.0% of the variance was accounted for by the three dopamine genes.

DISCUSSION

In attempting to find the gene or genes responsible for TS the majority of workers have focused almost exclusively on linkage studies using the model of autosomal dominant inheritance with reduced penetrance [Pakstis et al., 1991; Pauls et al., 1990; Heutink et al., 1992]. To date, despite "exclusion" of virtually 100% of the genome, this approach has been unproductive. A similar lack of success has been reported for most other psychiatric disorders. We have argued previously that TS is a polygenic spectrum disorder with genes being contributed by both parents [Comings, 1990a,b, 1994b, 1995d; Comings and Comings, 1992; Comings et al., 1989]. In polygenic disorders, the critical variable is the number of mutant genes present, rather than the consistent presence of a given mutant gene in affected members and its absence in unaffected members. Thus, when lod score linkage studies are attempted in a disorder that is actually polygenic, the presence of a given mutant gene in an unaffected member, who happens not to have a critical number of the relevant genes, will contribute to a negative lod score even though that gene may actually be involved in defining the phenotype [Comings, 1994d, 1996]. In addition, when a disorder is polygenically inherited, claims to have excluded a role of specific genes by linkage analysis, as in the case of the DRD2 [Devor et al., 1990; Gelernter et al., 1990], DβH, and DAT1 genes, no longer have validity.

We will refer to a single gene of a polygenic set as a *polygene* [Comings, 1996]. Association studies may be the only viable method of identifying the effect of polygenes. As the percentage of the phenotype accounted for by a polygene decreases, the difficulty of identifying that effect increases, and the number of subjects that must be studied also increases. One of the criticisms of association studies is that if the controls are unknowingly drawn from a different racial or ethnic group, the presence of differences in gene frequency in such groups may produce erroneous results. While this can theoretically be eliminated by using the haplotype relative risk technique [Falk and Rubinstein, 1987; Ott, 1989], as discussed in the introduction and elsewhere [Comings, 1996], for diallelic markers, where the frequency of the q allele is approximately .1, and where the gene being investigated accounts for less than 10% of the variance, the power of this technique is severely limited. In association studies, failure to carefully screen controls to exclude drug and alcohol abuse, as well as the condition being examined, can lead to false assumptions of no association.

The present study attempted to address all of these concerns. This involved the following: 1) All subjects were non-Hispanic Caucasians. 2) Not only were the controls screened for drug, alcohol, and tobacco abuse/dependence, they were assessed by the same structured instrument used for the TS probands and relatives. This permitted exclusion of controls that possessed the behavior being studied. The dramatic effect this had is illustrated by the fact that for the *total set,* the resulting variation in the prevalence of the D_2A1 allele in controls ranged from 35.0% to 23.8%, and the variation in number of eligible controls from 67 to 40 for the different behaviors. 3) A large number of subjects were examined to avoid type II errors. Thus, for the DRD2 locus 484 subjects were studied in the *total set* and 319 for the *polygenic set.* 4) All subjects were administered the same structured review of psychiatric symptoms, based on the DIS. TS is a complex spectrum disorder [Comings, 1990a, 1995e] and this allowed the examination of a number of different behaviors to test the possibility that the D_2A1 allele might be strongly associated with some behaviors but not others, rather than rely on a single dichotomous diagnostic entity (TS

TABLE XI. Comparison of the Behavior Score Means for Controls and TS Probands in the *Polygenic Set*

Behavior	Group[a]	Mean	S.D.	F ratio[e]	P*
ADHD	1	30.04	10.97		
	2	24.74	13.53		
	3	20.42[b]	13.77		
	4	14.07[b,c]	13.11	14.77	.0002
Stuttering	1	1.17	0.49		
	2	1.06	0.55		
	3	0.94	0.62		
	4	0.46[b,c,d]	0.64	14.61	.0002
ADHD-R	1	7.75	4.18		
	2	6.43	4.74		
	3	4.82[b]	4.53		
	4	3.53[b]	4.12	9.87	.0020
Oppositionally defiant	1	5.04	3.05		
	2	3.91	3.20		
	3	3.38	3.25		
	4	1.93[b]	2.84	9.56	.0023
Tics	1	4.95	4.41		
	2	3.71	3.30		
	3	3.28	3.65		
	4	1.40[b]	2.97	9.56	.0023
Conduct	1	4.08	2.51		
	2	3.05	2.35		
	3	2.87	2.23		
	4	1.93[b]	1.22	8.61	.0038
Obsessive-compulsive	1	3.37	3.51		
	2	3.02	3.03		
	3	2.75	2.91		
	4	1.13	1.99	5.48	.020
Mania	1	2.29	2.52		
	2	2.11	2.22		
	3	1.40	2.12		
	4	0.87	1.59	5.21	.024
Alcohol	1	1.21	3.45		
	2	0.35	1.70		
	3	0.20	1.07		
	4	0.00	0.00	4.50	.035
General anxiety	1	0.33	0.48		
	2	0.29	0.46		
	3	0.20	0.40		
	4	0.07	0.25	4.39	.038
Panic	1	3.45	2.39		
	2	3.37	2.44		
	3	2.97	2.15		
	4	2.13	1.12	3.79	.053
Schizoid	1	1.91	2.88		
	2	1.66	2.24		
	3	1.26	1.45		
	4	0.78	1.42	3.41	.067
Sleep	1	0.83	1.04		
	2	0.64	0.86		
	3	0.44	0.89		
	4	0.46	0.74	2.10	.149
Sexual	1	1.12	1.96		
	2	0.62	1.13		
	3	0.58	1.16		
	4	0.53	0.99	2.01	.158
Drugs	1	0.87	2.29		
	2	0.34	1.32		
	3	0.34	1.32		
	4	0.20	0.77	1.92	.168
Major depression	1	3.83	3.07		
	2	2.91	2.10		
	3	2.94	2.79		
	4	2.80	2.93	1.88	.173

(*continued*)

TABLE XI. Comparison of the Behavior Score Means for Controls and TS Probands in the *Polygenic Set (continued)*

Behavior	Group[a]	Mean	S.D.	F ratio[e]	P*
Learn	1	0.87	0.99		
	2	0.80	0.90		
	3	0.78	1.03		
	4	0.47	0.74	1.67	.197
Phobia	1	2.83	2.82		
	2	2.67	2.81		
	3	2.41	2.79		
	4	2.00	1.96	1.00	.318
Grade school	1	3.33	2.00		
	2	3.09	1.97		
	3	2.97	2.02		
	4	2.80	1.74	0.71	.399
Somatization	1	3.09	3.02		
	2	2.68	3.74		
	3	1.69	2.14		
	4	2.69	4.02	0.43	.525
Read	1	2.00	1.88		
	2	1.98	1.98		
	3	2.10	2.23		
	4	1.86	2.41	0.02	.893

*P value based on F-test.
[a]Group 1, D2A1+, DβHB1+, DAT1 10/10+ n = 24; Group 2, 2 of 3 + n = 67; Group 3, 1 or 3 + n = 70; Group 4, D2A1−, DBHB1−, DAT1 10/10− n = 15. Total = 176.
[b]Significantly different from group 1 at α = .05 by Tukey test.
[c]Significantly different from group 2 α = .05 by Tukey test.
[d]Significantly different from group 3 α = .05 by Tukey test.
[e]F-ratio by linear contrast for the four gene groups using ANOVA.

or not TS). 5) The inclusion of TS probands, TS relatives with and without TS, and controls, provided the opportunity for a much wider range of behavior scores to be examined. 6) To eliminate concerns about the inappropriate selection of controls, the results were also analyzed without including the controls. 7) The inclusion rather than purposeful exclusion of probands with comorbid conditions, since individuals may have a greater degree of genetic loading. 8) Collection of many more severely affected probands rather than focusing on the large families of the type used for linkage studies, where the non-proband patients are often more mildly affected. 9) The examination of the effect of combining two or more genes on the phenotype, in this case the *Taq* A1 allele of the DRD2 gene, the *Taq* B1 allele of the DβH gene, and the 10/10 genotype of the DAT1 gene. To accomplish this all three genes were tested in the same set of subjects.

Dopamine D_2 Receptor Gene

The D2A1 allele in TS probands versus other groups. As shown in Table II, for the total set, 41.7% of the TS probands carried the D_2A1 allele, vs. 35.0% for the relatives with TS, vs. 28.8% for the relatives without TS, vs. 26.9% for the controls without substance abuse disorders. This progression was significant at $P = .0038$. The results for our controls (26.9%) were indistinguishable from the prevalence of the D_2A1 allele of 25.9% in a total of 714 non-Hispanic controls screened to exclude alcohol and drug abuse/dependence [Comings et al., 1996c]. The prevalence in our TS probands (41.7%) was also virtually indistinguishable from that of the total of the 432 TS subjects genotyped by us and others, of 40.7%. While these results are consistent with a role of the DRD2 gene in TS, they do not define which part of the spectrum of behaviors are primarily affected. To determine that, we examined the prevalence of the D_2A1 allele in the three groups: *controls without, cases without,* and *cases with* the behaviors in question.

Twenty separate symptom clusters relating to impulsive, compulsive, addictive, affective, anxious, sleep, and learning behaviors were examined (Tables III and

TABLE XII. Mean Behavioral Scores for Some Behaviors by Whether All, Some, or None of the DRD2, DBH, and DAT1 Alleles Were Present

Gene combination	N	Mean	S.D.
ADHD Score			
D2+ DBH+ DAT1+	24	30.04	10.97
D2+ DBH+ DAT1−	22	25.04	12.72
D2− DBH+ DAT1+	34	25.91	14.83
D2+ DBH− DAT1+	11	20.54	10.75
D2+ DBH− DAT1−	10	21.50	12.90
D2− DBH+ DAT1−	43	19.96	13.20
D2− DBH− DAT1+	16	20.94	16.38
D2− DBH− DAT1−	14	14.07	12.10
Tic score			
D2+ DBH+ DAT1+	24	4.95	4.40
D2+ DBH+ DAT1−	22	4.00	3.18
D2− DBH+ DAT1+	34	3.91	3.59
D2+ DBH− DAT1+	11	2.54	2.54
D2+ DBH− DAT1−	10	5.00	4.96
D2− DBH+ DAT1−	44	2.84	3.31
D2− DBH− DAT1+	16	3.43	3.52
D2− DBH− DAT1−	15	1.40	2.97

TABLE XIII. Multiple Linear Regression Analysis and Proportion of the Variance Accounted for by the Three Dopamine Genes DRD2, DβH, and DAT1; Controls and TS Probands Only

	DRD21		DβH		DAT1			
Behavior scores	r	r^2	r	r^2	r	r^2	Total %[a]	Ratio[b] D2:DβH:DAT
Conduct	.217*	.047	.114	.013	.030	.0009	6.0	52:14:1
Mania	.197*	.039	.055	.0003	.084	.0007	5.0	130:1:2
Schizoid	.169*	.028	.046	.0021	.048	.0023	3.3	13:1:1
Grade school	.165*	.026	−.025	.0006	.0004	.0000	2.9	43:1:−
Tics	.155*	.024	.109	.0118	.108	.0116	4.7	2:1:1
OCD	.153*	.023	.040	.0016	.066	.0043	3.0	14:1:3
ODD	.149*	.022	.136	.0184	.108	.0117	5.2	2:2:1
Phobia	.149*	.022	−.041	.0016	.016	.0002	2.5	110:8:1
Sex	.142	.020	−.023	.0005	.061	.003	2.6	40:1:6
General anxiety	.141	.020	−.053	.0028	.180*	.032	5.9	7:1:11
ADHD	.125	.016	.166	.0275	.186	.0346	7.6	1:2:1
Panic	.123	.015	.004	.0000	.113	.0128	3.0	1:−:1
Drugs	.104	.011	.047	.0022	.024	.0006	1.4	18:3:1
Stuttering	.086	.007	−.005	.0000	.067	.0045	1.3	2:−:1
Somatization	.080	.006	.035	.0012	.075	.0056	1.4	5:1:5
Alcohol	.073	.005	.050	.0025	.160*	.025	3.4	2:1:10
Depression	.070	.005	−.023	.005	.183*	.033	4.1	1:1:7
Sleep	.041	.002	.088	.0077	.119	.014	2.3	4:1:7
Learn	−.013	.0002	.125	.0156	.040	.016	1.7	1:80:80
Read	−.063	.004	.127	.0161	−.049	.002	2.4	2:8:1
Total		.32		.11	.20			3:1:2

[a]Based on r^2 from multiple r for all three genes.
[b]Ratio of r^2 except for Total which is ratio of r.
*$P < .05$.

IV). (Twenty-one including the double assessment of ADHD.) The variables significantly associated with the D_2A1 allele were sexual, stuttering, obsessive-compulsive, schizoid, manic, ADHD-R, tics, ADHD, conduct, oppositional defiant, alcohol abuse, learning, and sleep problems. All other behaviors showed the same trend but were not significant. The results for the two most significant and least significant behavior for the *total set* are illustrated in Figure 1. Manic behaviors were the most significant for chi square studies of the *polygenic set* and ranked high on the tables. This is consistent with our studies of the behaviors in relatives of TS probands suggesting that manic symptoms represented the highest form of expression of the *Gts* genes [Comings, 1995e].

A significant role of abnormalities in dopamine metabolism have been implicated in several of the most significant behaviors, i.e., sexual disorders [Gessa and Tagiamonte, 1975], mania [Goodwin and Jamison, 1990], schizoid behaviors [Carlsson, 1978; Snyder, 1976], ADHD [Shaywitz et al., 1976], conduct disorder or aggression [Rogeness et al., 1986; Valzelli, 1981; King, 1986], alcohol abuse [Blum et al., 1990], and stuttering. A role of dopaminergic abnormalities in the latter is suggested by the fact that dopamine plays a major role in fine motor movements and speech requires the coordination of many small muscles. In addition, haloperidol, a DRD2 receptor antagonist, has been reported to be effective in the treatment of some stutterers [Murray et al., 1977; Prins et al., 1980]. While serotonergic mechanisms have been most often implicated for obsessive-compulsive behaviors, abnormalities in dopamine have also been considered [Austin et al., 1991; Delgado et al., 1990]. Abnormal circuits involving the thalamus, basal ganglion and frontal lobes have been implicated in obsessive-compulsive disorder [Baxter et al., 1992; Rauch et al., 1994; Modell et al., 1989] and dopamine is a major neurotransmitter especially in the striatum and frontal lobes.

The general lack of an association between major depressive episode and the DRD2 gene is consistent with

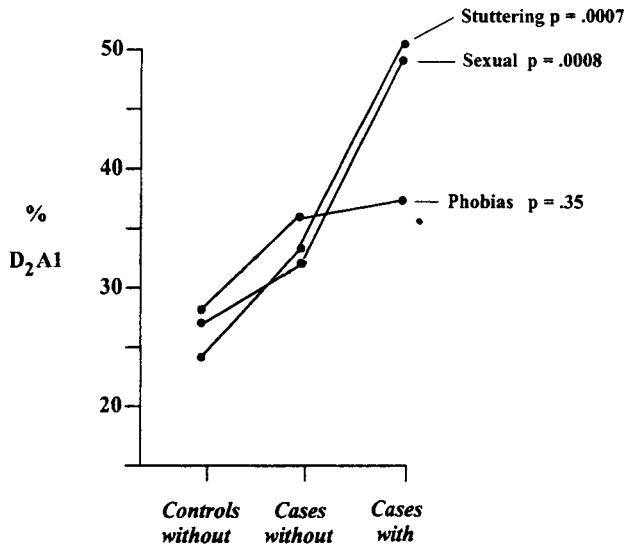

Fig. 1. Comparison of the prevalence of the D_2A1 allele for the *total set* in a) controls without substance abuse or the behavior in question (*controls without*), b) TS probands or relatives without the behavior in question (*Cases without*), and c) TS probands or relatives with the behavior in question (*Cases with*), for the two most significant behaviors and least significant behavior.

our studies of pathological gambling [Comings et al., 1996c]. We found that 50.9% of 151 non-Hispanic Caucasian pathological gamblers carried the D_2A1 allele vs. 25.9% of 714 non-Hispanic Caucasian controls screened for drug or alcohol abuse ($P = 10^{-8}$). Among 17 female pathological gamblers, of the 11 that had a history of comorbid major depressive episode, only 9.1% carried the D_2A1 allele, while of the 6 that had no history of a major depressive episode, 83.3% carried the D_2A1 allele.

The lack of association between the presence of the D_2A1 allele and the severity of tics when only TS probands are examined, agrees with the results of Devor [1992] and Gelernter et al. [1994b]. However, when controls and non-TS relatives were included, the tic score was significant. In addition, when cases were stratified by other behaviors the D_2A1 allele was associated with many of them.

Dopamine β-Hydroxylase

The prevalence of the DβH *Taq* B1 allele was modestly, but significantly higher in 352 TS probands (70.5%) than in 148 controls (60.8%). The only other group of subjects that showed a significantly elevated prevalence of the B1 allele was smokers at 73.1%.

For the *polygenic set* of 319 subjects the 13 variables significantly associated with the DβH B1 allele were, in order, ADHD, learn, grade school, ADHD-R, oppositional defiant, tics, mania, alcohol, reading, drug abuse, sleep, stutter, and obsessive-compulsive. However, for several of these, especially those of borderline significance (alcohol, drug abuse, stutter, and obsessive-compulsive), the prevalence of the B1 allele was higher in the TS probands and relatives without the behavior than in those with the behavior, suggesting the *P* values were being driven by the lower frequencies in the controls. The results were similar for the larger *total set* of 455 subjects except for the addition of somatization and major depression and the non-significance of stuttering.

For the *polygenic set,* the prevalence of the B1 allele in controls ranged from 46.9 to 56.5%. When the means of the behavioral scores were examined for controls and TS probands, with both the *polygenic set* and the *total set,* ADHD, grade school, oppositional defiant, and sleep were significant. These results are consistent with the literature reports of an association between serum or CSF DβH and ADHD, conduct and learning disorders (see Introduction). The interesting difference is that in our studies oppositional defiant behavior was consistently more significantly associated with the B1 allele than conduct disorder.

Studies correlating serum DβH levels with genotyping need to be completed to determine with certainty whether the levels in B1 subjects are associated with a decrease, or increase, in serum levels. Like the DRD2 A1 allele, the DβH B1 allele was most associated with the variables ADHD, obsessive-compulsive, manic, oppositional defiant, and sleep. Differences included the greater association of the DRD2 A1 allele with the schizoid, sexual conduct, and stuttering variables, while the DβH B1 allele was more strongly associated with learning, reading, and school problems. The tendency for the variables associated with school performance, such as reading, learning, and grade school, to rank high in the DβH studies is especially striking in contrast to the DRD2 studies where they tended to rank at the bottom. This may be related to the role of DβH in memory (see Introduction).

It is of interest that some cases of orthostatic hypotension with absent norepinephrine metabolites epinephrine and 3-methoxy-4-hydroxyphenyl glycol, and high levels of dopamine and dopamine metabolites 3,4-dihydroxy phenylacetic acid, and homovanillic acid, are associated with an absence of plasma DβH [Gary and Robertson, 1994]. Surprisingly, these patients appear to be generally normal or near normal in their mood and mental status. Whether the deficiency extends to CNS DβH levels has not been reported. While further studies will be required, it is possible that the difference may be that a mild deficiency of DβH, such as that assumed to be present in the TS patients, results in a primary norepinephrine deficiency with no significant increase in dopamine levels, while severe DβH with a dramatic increase in dopamine levels may have a different phenotype.

Dopamine Transporter

The cloning, sequencing and identification of the 5′ UTR 40 bp repeat polymorphism in the DAT1 gene allowed the examination of the potential role of the genetic variants of this important transporter in various psychiatric and behavioral disorders. In this regard, Persico et al. [1993] examined this polymorphism in subjects with drug abuse and found no significant difference in the frequency of any of the 3′ UTR repeat alleles compared to normal controls. Recently Gelernter et al. [1994a] reported an association between the 9 allele of the 40 bp repeat of the DAT1 gene with cocaine induced paranoia. Among their 56 cocaine addicts with a history of cocaine induced paranoia, the frequency of the 9 allele was .30 compared to a frequency of .15 for their 47 cocaine addicts without cocaine induced paranoia ($P = .014$). They found no difference in the frequency of the 9 and 10 alleles in Whites vs. Blacks.

Studying postmortem samples of TS subjects, Singer et al. [1991] reported an increased number of dopamine uptake sites in the striatum suggesting either a greater number of DAT1 molecules or an increased number of dopamine nerve terminals. It is not known whether the less common DAT1 repeat alleles are associated with an increase or decrease in the number of DAT1 molecules.

The significant increase in prevalence of the 10/10 genotype in TS probands suggested that alleles of the DAT1 gene were contributing to the TS phenotype. The significant increase in 36 subjects with autism is consistent with studies suggesting that TS and autism are genetically related and involve similar sets of genes [Burd et al., 1987; Comings and Comings, 1991b; Sverd, 1991].

To further examine the possible role of the DAT1 gene, we compared the prevalence of the 10/10 genotype for different behaviors in controls, TS probands, and relatives of TS subjects, as in the studies of the DRD2 and DβH genes. This showed a significant association with a number of variables for the comorbid behaviors in-

cluding somatization, alcohol dependence, ADHD, major depression, obsessive-compulsive, general anxiety, manic, sexual, and oppositional defiant disorder.

While the present results support those of Gelernter et al. [1994a] showing an apparent physiological effect associated with alleles of the 40 bp repeat of the DAT1 gene, our results also suggest that the psychopathology is associated with the 10 allele rather than with the 9 allele. This was further supported by the finding that in every behavior that showed a significant effect, there was a progressive decrease in the frequency of the 9 genotype (table not shown). For example, for somatization the frequency of the 9 allele decreased from .37 in the controls to .29 in the relatives without somatization, to .20 in the relatives with somatization, while the frequency of the 10 allele increased from .54 to .70 to .77 across the same groups. It is of interest that the frequency of the most common allele, 10, appears to increase still further in the presence of various sets of behavioral symptoms. One possible explanation is that the 9 allele was originally the normal allele and the 10 allele has increased in frequency by selection because of its association with one or more of the listed behaviors.

After this manuscript was submitted for review, Cook et al. [1995] also reported an association between the 10 alleles of the DAT1 gene and ADHD in 57 cases of ADHD, and Malison et al. [1995] reported abnormalities in the striatal dopamine transporter by SPECT studies. Gelernter [1995] reported the association of another dopamine gene, the dopamine DRD4 receptor, with Tourette syndrome, despite negative results using linkage.

All Three Dopaminergic Genes

The above studies showed that each gene, when examined individually, showed a significant but modest effect on many of the same behavioral variables. To study the additive and subtractive effects required that we obtain DNA samples on a significant number of relatively severely affected TS probands, their relatives, and controls, and that the same subjects be genotyped for all genes. This was done with 319 samples. An a priori assumption was one of two strategies would provide the more power. The first strategy involved examining the whole *polygenic set,* including largely unaffected relatives, to increase the power. The second strategy was to examine a sub-portion of the *polygenic set* consisting only of controls and TS probands. This would provide the widest range of scores and a greater dichotomization by severity. While both techniques gave positive results, the latter proved to be the more effective.

The results showed that for all but four of the behaviors examined (somatization, major depression, sleep and reading), there was a linear decrease in scores progressing from subjects that carried 3 of the 3 markers, to those with 2 of 3, 1 of 3, and 0 of 3. This is consistent with our a priori hypothesis of a progressive decrease in means across these groups, and our hypothesis that a wide range of psychiatric behaviors share genes in common and that once the dopamine-serotonin and other neurotransmitter balance is upset, the resulting brain dysfunction can result in a wide range of different behaviors [Comings, 1990a, 1994b, 1995e; Comings and Comings, 1991a]. Put in other terms, instead of following the one-gene, one-disorder model, psychiatric disorders are multifactorial (partly environmental), polygenic, spectrum disorders.

We have repeatedly argued that TS is a spectrum disorder and the *Gts* genes are responsible for each of its many associated behaviors [Comings, 1990a; Comings and Comings, 1991a, 1993; Comings, 1994a,b, 1995a,e]. We have also argued that TS and ADHD are fundamentally the same genetic disorder [Comings and Comings, 1984, 1987b, 1993; Knell and Comings, 1993]. The demonstration that when these three dopaminergic genes were combined, two of the behaviors that were most significant were ADHD and tics, provides confirmation for this hypothesis at a molecular genetic level. The significant correlation with other behaviors supports the concept that they constituted a spectrum of genetically interrelated behaviors. We presume that numerous other genes will be added to the list and that some will tip the phenotype in directions less influenced by dopaminergic genes. The tryptophan 2,3-dioxygenase gene, with its effect on serotonin levels, is one example [Comings et al., 1996b].

When the behavior most significantly associated with these three genes, ADHD, was examined in more detail, by listing all possible combinations of the three markers, these results were also consistent with an additive and subtractive effect. This is illustrated in Figure 2. The most densely shaded area shows the part of the ADHD score that is diagnostic for ADHD, the lightly shaded area is a borderline transition region and subjects scoring in the unshaded area have too few symptoms to diagnose the disorder. This shows that the loading for three genes can account for a range of clinically significant scores from no diagnosis of ADHD to an unambiguous diagnosis. This figure, however, should not be interpreted to indicate that there is now a specific genetic test for ADHD or TS, or the other related behaviors. The story is still incomplete and other genes will be found to contribute. For example, there are still some subjects with all three markers who had no symptoms of ADHD, and some with none if the three markers who had clear cut ADHD. This is an expected part of the multifactorial, polygenic nature of psychiatric disorders.

Not all of the behavioral scores showed this degree of correlation with each of the eight permutations. To illustrate this, the results for the same type of dichotomization is shown in Table XII for the tic score. While there is again an approximate and significant linear decrease in tic score across the different permutations, subjects positive for the D_2A1 allele, but negative for the DβH B1 and DAT1 10/10 genotype, presented a notable discontinuity with a mean score of 5.0. Whether this is a statistical aberration due to the relatively small number of subjects in each group, or an indication that the DRD2 allele has a stronger effect on tics than the other genes, must await further studies. The studies of the DRD2 gene by itself, showed a significant association with tic severity.

Some of the other results in Table XI deserve comment.

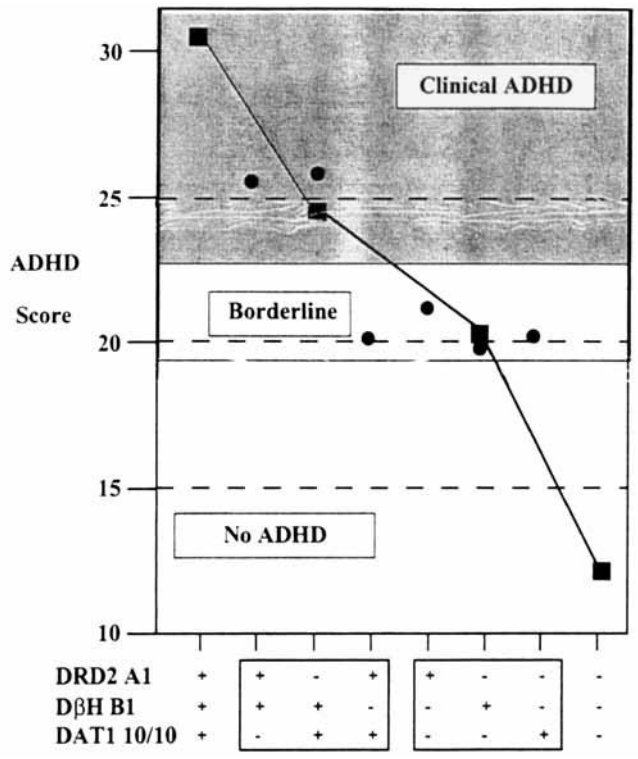

Fig. 2. Diagram of the mean ADHD score for various combinations of the DRD2 *Taq* A1, DβH *Taq* B1, and DAT1 10/10 alleles. The means for the major classifications 3 of 3, 2 of 3, 1 of 3, and 0 of 3 are shown as black squares. The minor classifications, delineated at the bottom of the figure, are shown as black circles.

For example, the genes responsible for alcoholism are unknown, although the many studies to date support a significant role for the DRD2 gene in more severe cases [Noble, 1993; Blum et al., 1995]. Even though this was not a study of alcoholism, and even though none of the subjects were ascertained because of alcohol problems, the linear progression was significant, suggesting that these three genes in combination deserve further study in a population of alcoholics and controls.

In previous studies we have shown a significant increase in the frequency of stuttering in TS probands [Comings and Comings, 1987b] and their relatives with TS [Comings, 1995e], and have suggested that stuttering is another manifestation of the *Gts* genes. The present finding that stuttering ranked just below ADHD and higher than tics per se, support this proposal.

We have recently demonstrated a highly significant increase in conduct and oppositional defiant disorder with increased genetic loading for *Gts* and ADHD genes [Comings, 1995d]. The present molecular genetic studies strongly support these results. These observations stand in contrast the generally held assumption that these two behavioral disorders are entirely due to psychosocial factors, including poor parenting. While no one can doubt the critically important role of competent parenting, genetic factors may play the major role in conduct and oppositional defiant disorder when parenting styles or environmental factors are not at fault.

Proportion of the Variance Accounted for by the Dopamine Genes

Calculation of the correlation coefficients using multiple linear regression analysis allowed an estimation of the proportion of the variance for the different behaviors accounted for by these three dopaminergic genes. For all three genes this ranged from 7.6% of the variance for the ADHD score to 1.3% of the variance for stuttering (Table XIII). To obtain an estimate of the relative importance of the three genes, the r^2 value was summed across all the behavioral variables. This suggested the relative importance of the three genes was in the approximate ratio of 3:2:1 for the DRD2, DAT1, and DβH genes, respectively. This conclusion is supported by the fact that for the DRD2 gene, r was significant for eight of the behavioral variables (conduct, mania, schizoid, grade school, tics, OCD, ODD, and phobia), for the DAT1 gene, r was significant for three of the variables (depression, general anxiety, and alcohol abuse). While for the DβH gene r was significant for none of the variables, the five with the highest values were ADHD, ODD, conduct, reading, and learning disorders.

These results also provide support for the concept that different genes and combinations of genes play a role in how the phenotype will be expressed [Comings, 1995f]. For example, all three genes are about equally involved in ADHD and ODD, while in conduct disorder the involvement is DRD2 > DβH > DAT1. For learning and reading, the order was DβH > DAT > DRD2.

Since the concordance rate for chronic tics in identical twins is less than 100% [Price et al., 1985] a portion of the variance (about 10 to 20%) is due to environmental factors. Since the *Taq* polymorphisms used for the DRD2 and DβH gene are not the sequence changes responsible for the functional variations in the genes, but are only in linkage disequilibrium with the functional mutations, these estimates of the percent of variance involved are probably underestimated, i.e., they would be higher if the functional mutations themselves were being tested. Finally, these estimates represent an average across all cases, controls and TS probands, whether they had the behavior in question or not. For example, as shown in Table III, only 41 of the 282 *cases* or 14.5% had problems with stuttering, while 43.7% had conduct problems. The percent of the variance accounted for by those who actually had the behaviors in question, is probably much higher.

The results shown in Table XIII are consistent with the polygenic, polyfactorial nature of these disorders. The relatively low proportion of the variance accounted for by these three genes, despite the significant associations for *controls without, cases without,* and *cases with,* provides insight into why standard linkage analyses have been unproductive, and how sensitive this association approach is for polygenes.

Ethnic Stratification

One of the most common objections to the association technique is the concern that the results are actually due to a difference in the frequency of the genetic marker being tested in different racial or ethnic groups,

combined with a disproportionate number of one of these ethnic groups in the controls or the subjects. The following are reasons we believe this is unlikely in the present studies.

1) All subjects were restricted to non-Hispanic Caucasians of northern or western European ancestry.

2) The ethnic background of each subject was determined in all four grandparents. The number of different ancestral groups ranged from 1 to 12 with most subjects having 4 to 6 different ancestral groups.

3) For the D2A1 allele, a total of 13 different studies have examined 714 controls screened to exclude alcohol and drug abuse. The prevalence of the D2A1 allele in these studies averaged 25.9% and ranged from 12.5 to 34.8%, all lower than the 40.7% in the 432 TS cases.

4) When the mean behavioral scores were examined in D2A1 carriers versus non-carriers, several of the means were significant even when the controls were excluded from the analysis. The same was true for the combined examination of all three dopamine genes.

5) Different subjects tended to score high on different behavioral scores. Despite this, there were many significant results when the three groups of *controls without, cases without,* and *cases with* were examined. The chance of all these results actually being the result of hidden ethnic stratification rather than stratification by presence or absence of the variable being examined, seems remote.

6) Positive results were obtained with all three of the dopamine genes examined, using the same *polygenic set* of controls and subjects. The likelihood that hidden ethnic stratification was involved in all three of these genes seems remote.

7) These positive results are not simply a result of the fact that multiple behavioral variables are being examined, and thus a few should be significant by chance. We have obtained totally non-significant results for all the behavioral variables for several other genes tested.

Implications for Psychiatric Genetics

These studies have a number of implications for psychiatric genetics.

Polygenic inheritance. We believe these results are consistent with a polygenic mode of inheritance of TS in which different common genetic variants play an additive role in defining the phenotype, and the different genes involved may weigh the phenotype more heavily in a specific direction. Thus, those involving dopamine may be somewhat more likely to present with ADHD, schizoid and sexual behaviors, stuttering, tics, substance abuse, conduct and oppositional defiant disorders than with major depression or the anxiety disorders. Other polygenes of the set, such as those involving serotonin, may slightly push the phenotype in a different direction, such as toward depression or anxiety.

Spectrum disorders. These results indicate that individual genes such as the DRD2, DβH, and DAT1 genes can play a role in a wide spectrum of behaviors. We have argued elsewhere that TS and other psychiatric disorders are spectrum disorders associated with a wide range of behavioral problems [Comings, 1990a, 1994a,b, 1995a,d,e; Comings and Comings, 1987, 1993]. One of the most convincing demonstrations of the concept of spectrum disorders would be the observation that at a molecular level genetic variants of the same set of genes were associated with a wide range of different behavioral disorders. The present observations of a significant correlation between the presence of specific markers of the DRD2, DβH, and DAT1 genes and a variety of different behaviors supports this concept and is consistent with our proposal that the most important result of the combined set of polygenes is the disruption of the dopamine–serotonin (and other) neurotransmitter balance, which eventuates in a spectrum of disturbed behaviors [Comings, 1994a,b; Comings and Comings, 1992].

The need to include relatives and controls in the analysis. If we had examined only TS probands, many of these associations would not have been significant. In fact, when limited to the 225 TS probands, only the conduct, somatization, and stuttering variables were significantly associated with the D_2A1 allele. Limiting the study to TS probands limits the range of the scores and as a result reduces the chance of observing a significant correlation with the DRD2 genotype. This truncation of the range of scores is well illustrated by the ADHD score. Thus, when the controls, relatives and TS probands were examined, the mean score for those with the D_2A1 allele was 20.60 vs. 16.68 for those without the D_2A1 allele ($P = .003$). By contrast, when only the TS probands were examined the mean for the D_2A1 allele carriers was 27.53 vs. 27.34 for the non-D_2A1 carriers ($P = .90$). The same point is valid if only normal subjects without psychopathology were examined. By analogy a positive correlation between education level and income might be missed if only CEO's of the Fortune 500 were examined. If a significant range of a given behavior score is not present in the group being studied, whether controls or probands, a correlation between a behavior and a given polymorphism may be missed. Studies of TS (or other) probands only, may produce falsely negative data.

Expected high frequency of marker alleles in unaffected relatives. One of the expectations of polygenic inheritance is that the frequency of an allele of a gene that plays a role in the expression of the phenotype can be almost as high (or sometimes higher) in the unaffected relatives as in the probands. This is because in polygenic inheritance the critical factor is the clustering together of a number of genes, not the co-segregation of the disorder with a specific gene. Thus, the most sensitive technique for detecting the role of a polygene is to compare the frequency of a marker allele in probands vs. unrelated controls. By contrast, in autosomal dominant, single gene inheritance, the frequency of the disease gene mutation (or marker of the mutation) approaches 100% in affected probands and 0% in unaffected relatives. Failure to appreciate this difference between polygenic vs. single gene inheritance can lead to the false assumption that a high prevalence of a marker allele (such as the D_2A1 allele) in unaffected relatives, rules out a role of the gene (such as the DRD2 gene) in the phenotypic expression of a disorder (such as TS [Gelernter et al., 1995]).

Need to carefully study controls. Many association studies give little or no information on the controls. We find it is just as important to screen the controls for a wide range of psychiatric disorders as the probands and their relatives. The importance of this is illustrated in the present study, which showed decreases in the prevalence of the alleles being examined when controls with alcohol, drug and tobacco dependence, and the different behaviors, were excluded.

Advantages of studying correlations with specific behaviors. Since each subject in the study has a different mix of the various behaviors, and all the behaviors showed the same trend, and most were significant at $\alpha = .05$, the examination of the prevalence of the marker alleles by specific behaviors largely eliminates one of the major concerns of association studies, i.e., that the results are simply due to ethnic differences between the controls and the TS families. It is extremely unlikely that some hidden confounding ethnic stratification would continue to hold over such a wide range of different significant correlations. This is especially true for the studies comparing the mean behavioral scores in D_2A1 versus D_2A2A2 groups using the t-statistic, since the relatively small group of controls were mixed in with all the other subjects and not examined separately. A further indication that these results are not due to a hidden difference between the controls and subjects is that the prevalence of the D_2A1 allele in our study was virtually identical with that of the 714 non-Hispanic, non-alcoholic, or drug addicted subjects in the literature, and that similar t-statistics were obtained when the controls were eliminated entirely.

Effect of comorbid disorders. While the reports of an association between alcoholism and the DRD2 allele have been quite variable, four of four reports to date indicate an association between the D_2A1 allele and polysubstance/drug abuse [Smith et al., 1992; Noble et al., 1993; O'Hara et al., 1993; Comings et al., 1994]. Despite this, in the present study, drug abuse was not significantly associated with the presence of the D_2A1 allele. However, the increased prevalence of the D_2A1 allele in individuals in the general population with drug abuse, may in large part be because they have ADHD, conduct, oppositional defiant disorder or TS [Comings, 1994b], each of which is associated with the D_2A1 allele. In a group of subjects such as TS probands, where the prevalence of ADHD, conduct disorder, oppositional defiant disorder is even higher than in population based subjects with drug abuse, the correlation of the D_2A1 allele with the drug abuse variable may be diluted or masked. Thus, the association between a specific gene and a given disorder may be dependent upon both the disorder being studied, and the company it keeps.

Need to study large numbers of subjects. The use of a relatively large number of subjects is important when testing for the effects of a polygene that may account for less than 10%, and possibly only 1 to 2% of the variance.

Correlation by specific behaviors may be the most powerful approach for psychiatric genetics. The above considerations, plus the fact that linkage studies, whether by lod score, sib pair analysis, or the haplotype relative risk technique, may be too inefficient for identifying the effect of polygenes, suggests that the present approach of examining the effect of a gene in question on the degree of expression of a number of specific behavioral variables may be the most powerful technique available for psychiatric genetics. This is supported by our results with the dopamine β-hydroxylase and dopamine transporter where there were minimal or no significant differences in allele frequencies or prevalences between controls and TS subjects, but highly significant differences for some of the individual behaviors.

Expected variability of results. The nature of polygenic inheritance, and the low percentage of variance accounted for by a single gene, suggests that different sets of genes will be involved in different subjects, different studies, and different sets of patients performed in different geographical locations. Thus, significant variations between different studies should be the rule rather than the exception. For example, one study done in Germany showed no association between the D_2A1 allele and alcoholism [Schwab et al., 1991] while a study done in a neighboring country, France, showed a striking difference in D_2A1 frequency in controls versus alcoholics [Amadeo et al., 1993]. The final truth about the role of a given gene in a given disorder, or a given behavior, may have to await the completion of a number of studies followed by a metanalysis of the total set [Schmidt, 1992].

Non-exon mutations and polygenes. All of the exons of the DRD2 gene have been extensively searched for mutations affecting gene function, with essentially negative results [Gejman et al., 1994; Sarkar et al., 1991]. This has been used by some to imply that there are no functionally significant allelomorphic variants of the DRD2 gene and thus all purported associations are invalid [Gelernter et al., 1994b]. We have also found no functional mutations in any of the 12 exons of the tryptophan 2,3-dioxygenase gene [Comings et al., 1996a], despite the significant association between intron polymorphisms with specific disorders [Comings et al., 1996b]. As pointed out elsewhere [Comings, 1996], one of the distinguishing characteristics of polygenes is that they are common in the population. Because of this the risk that a given individual may by chance inherit a number of such genes is high and the prevalence of polygenic disorders (.1 to 20+%) is much higher than for single gene disorders (<.1%). The reason that these mutations can be so common is that the mutations involved cause only minor to modest decreases in gene function. For example, in the case of the DRD2 gene, D_2A1 carriers showed only a 30% decrease in receptor density compared to D_2A2 carriers [Noble et al., 1991]. This suggests that the type of mutations involved may be fundamentally different for polygenes than for single gene disorders, and may preferentially involve introns, or 5′ regulatory sequences or 3′ sequences [Comings, 1995] possibly at great distances from the exons. As such these mutations may be extremely difficult to identify and writing the obituaries on their involvement in psychiatric disorders because of an absence of exon mutations, may be premature.

Additive and subtractive effect of polygenes. The ultimate proof of polygenic inheritance of neuropsychiatric disorders would be to show that several mutant alleles are additive in their effect on the phenotype. We propose that this has been demonstrated to occur for three dopaminergic genes, DRD2, DβH, and DAT1. Karp [1994] has stated that "currently available methods of pedigree analysis do not have great power to resolve the individual genetic factors contributing to a polygenically determined disorder." We believe the methods detailed in this manuscript provide that power.

ACKNOWLEDGMENTS

This work was supported in part by the National Institutes of Drug Abuse grant RO1-DA08417 and Tobacco Related Research Disease Program grant 4RT-0110.

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